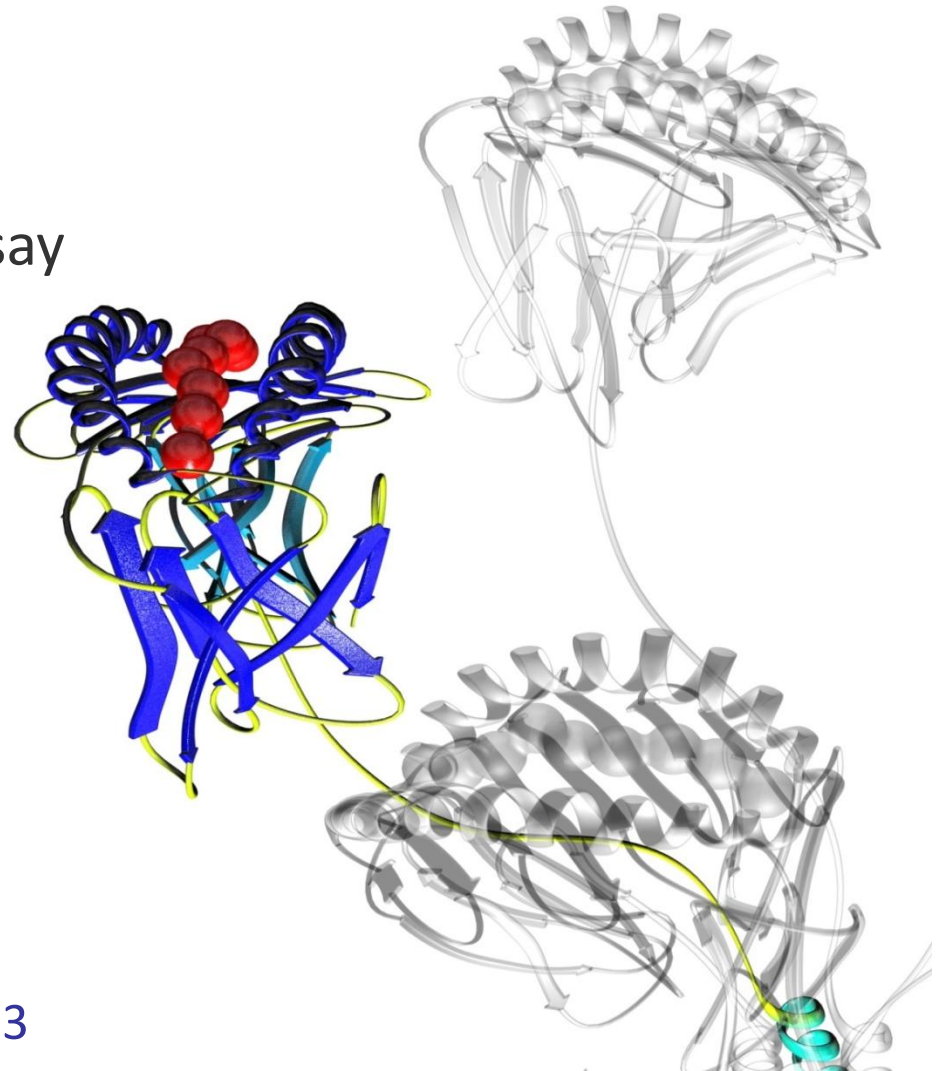


Mastering Immunity

ProPresent®

Antigen Presentation Assay

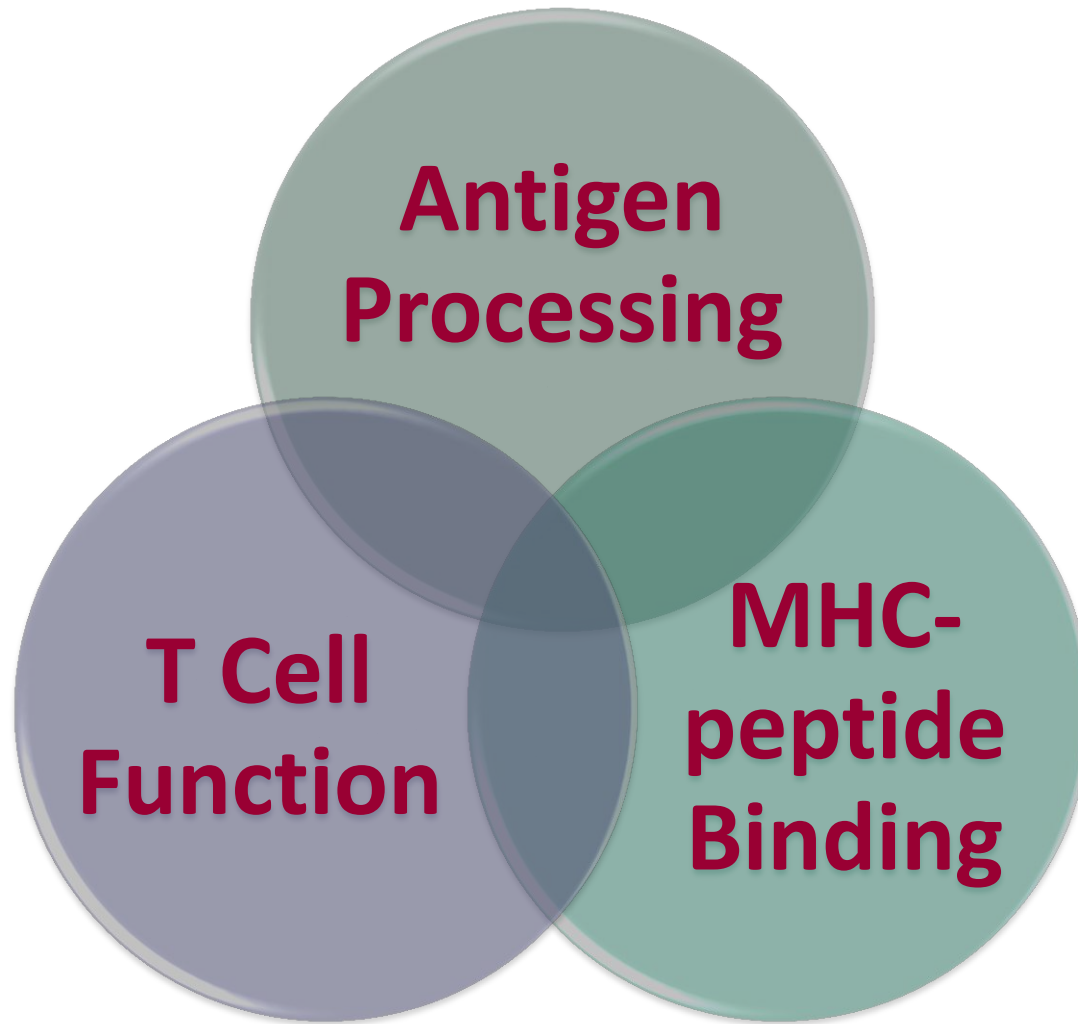


Nikolai Schwabe, CEO

EIP Symposium: Munich 2013



T Cell Epitope Antigenicity Profiling



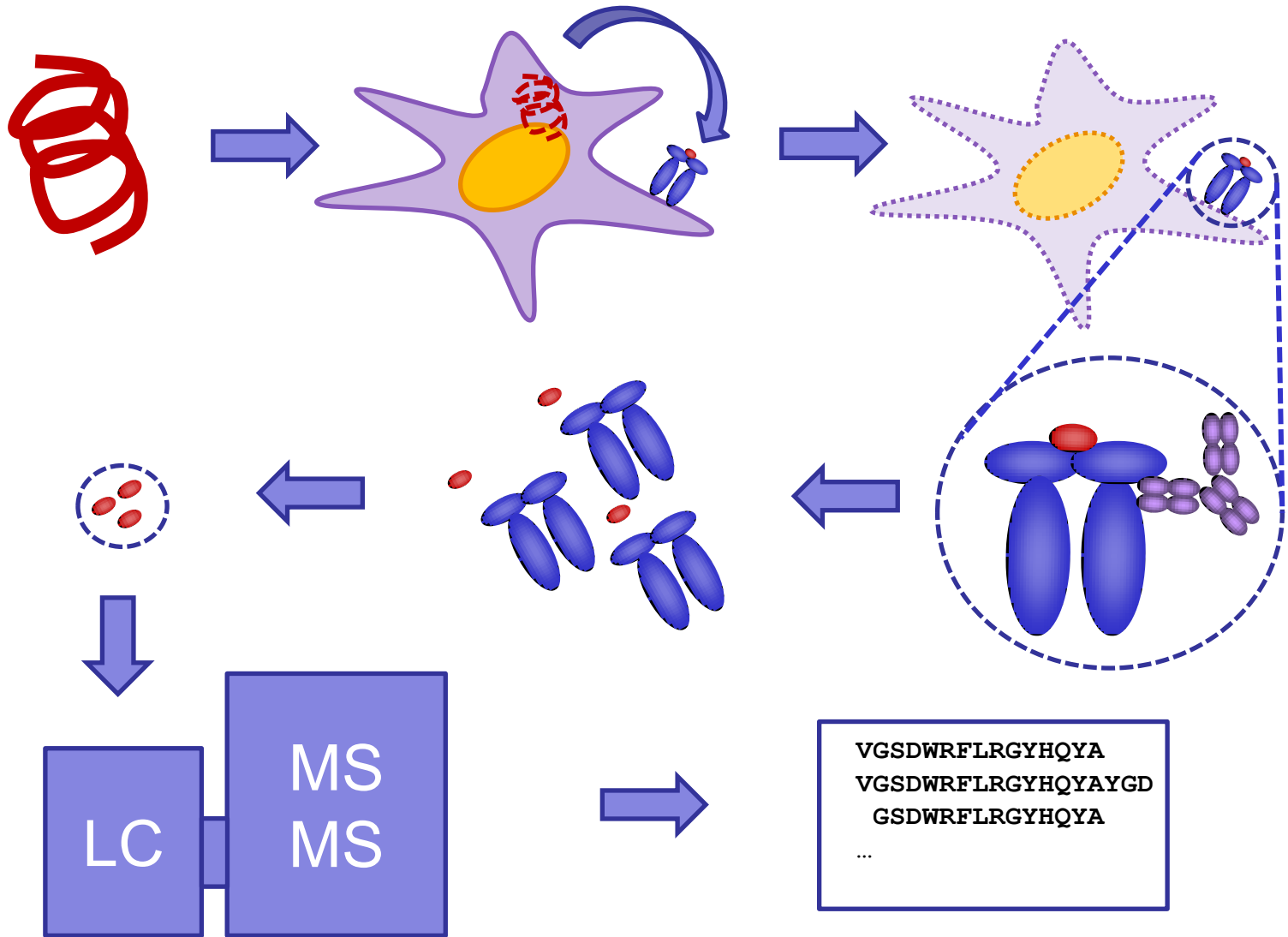
ProPresent[®] - a Flexible Service

(since September 2011)

- ProPresent[®] directly measures MHC-peptide presentation on DCs cultured with protein of interest
- Cellular *in vitro* assay, carried out on a set of HLA typed donor samples provided by ProImmune
- The only broad-based commercial service for this purpose
- Currently available for HLA-DR, DP, DQ, Class I HLA; can also be done for other species
- Rapid service: e.g. 2-3 antigens can be tested on 10 donors each in just **3-4** weeks



ProPresent Workflow



Applications of ProPresent®

- Final confirmation that sequences can be presented
- Key tool for understanding immunogenicity
 - Evaluating the impact of protein modifications (post translational, sequence variants, haptens, damage)
 - Impact of allelic variants of proteins (can be very important for replacement factors)
 - Impact on aggregation?
 - Presentation on different cell types?
 - Presented host-cell protein content?



Detection of Control Proteins

Donor ID	CLIP	LAMP-3	TFRC	FcER2/FcGR2	Apolipoprotein B	ITGAM
1	Yes	Yes	Yes	Yes	Yes	Yes
2	No	No	Yes	No	Yes	Yes
3	No	Yes	Yes	Yes	Yes	Yes
4	Yes	Yes	Yes	Yes	Yes	Yes
5	No	Yes	Yes	Yes	Yes	No
6	Yes	Yes	Yes	Yes	Yes	Yes
7	No	Yes	Yes	Yes	Yes	Yes
8	No	Yes	Yes	yes	Yes	Yes
9	Yes	No	Yes	No	Yes	Yes
10	No	Yes	Yes	Yes	Yes	Yes



Case Study: Presentation of KLH

Donor	DRB1_1	DRB1_2	Sequence	Protein Domain	Amino Acid Start/End	Expect Value
1	*03:01	*07:01	LPSLINDATYFNSRSQTFDPNPF	KLH-2	1372-1394	0.091
			SLINDATYFNSRSQTFDPNPF	KLH-2	1374-1393	0.021
			INDATYFNSRSQTFDPNPF	KLH-2	1376-1394	0.0038
2	*12:01	*08:01	SSDEVLALEKALDD*	KLH-2	32-45	0.000078
			SSDEVLALEKALDDLQ	KLH-2	32-47	0.001
			SSDEVLALEKALDDLQQ	KLH-2	32-48	0.025
			-----	-----	-----	-----
			VGDNFFLKYEAFDL	KLH-2	1226-1239	0.023
			VGDNFFLKYEAFDLNG	KLH-2	1226-1241	0.047
			VGDNFFLKYEAFDLNGG	KLH-2	1226-1242	0.06
-----	-----	-----	-----			
YDDTFTIKVHIKDIAG	KLH-2	2058-2073	0.039			

Excerpt of KLH peptides identified

Nested sequences are identified by dashed (----) lines between nested sets for each donor sample

Peptides marked with * have detectable modifications

Expect values ≤ 0.05 are indicative of peptide identity; currently accepted stringency criterion

Expect values < 0.3 are indicative of peptide homology: expect values of ≥ 0.05 are indicated by shaded areas

False Discovery Rates are $< 1\%$ for ProPresent™

Case Study: Humira®

Immunogenicity incidence is predominantly measured in clinical trials and in some (usually limited) post marketing follow-up studies

- The label for Humira® indicates that the incidence of ADA has been measured at 1-12%
- A recent publication shows that it can be more than 50% after 28 weeks of treatment (van Schouwenburg *et al.*, Ann Rheum Dis 2013;72:104-109 doi:10.1136/annrheumdis-2012-201445)

Alignment of detected Humira® peptides to CDR regions

Variable heavy domain (122 amino acids)

Regions: CDR-H1 CDR-H2 CDR-H3

.....|<----->|.....|<----->|.....|<----->|.....

1 10 20 30 40 50 60 70 80 90 100 110 120

EVQLVESGGGLVQPGRSLRLSCAASGFTFSDDYAMHWVRQAPGKLEWVSAITWNSGHI DYADSV EGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAKVS YLSTASSLDYWGQGTTLVTVSS

Variable light domain (107 amino acids)

Regions: CDR-L1 CDR-L2 CDR-L3

.....|<----->|.....|<----->|.....|<----->|.....

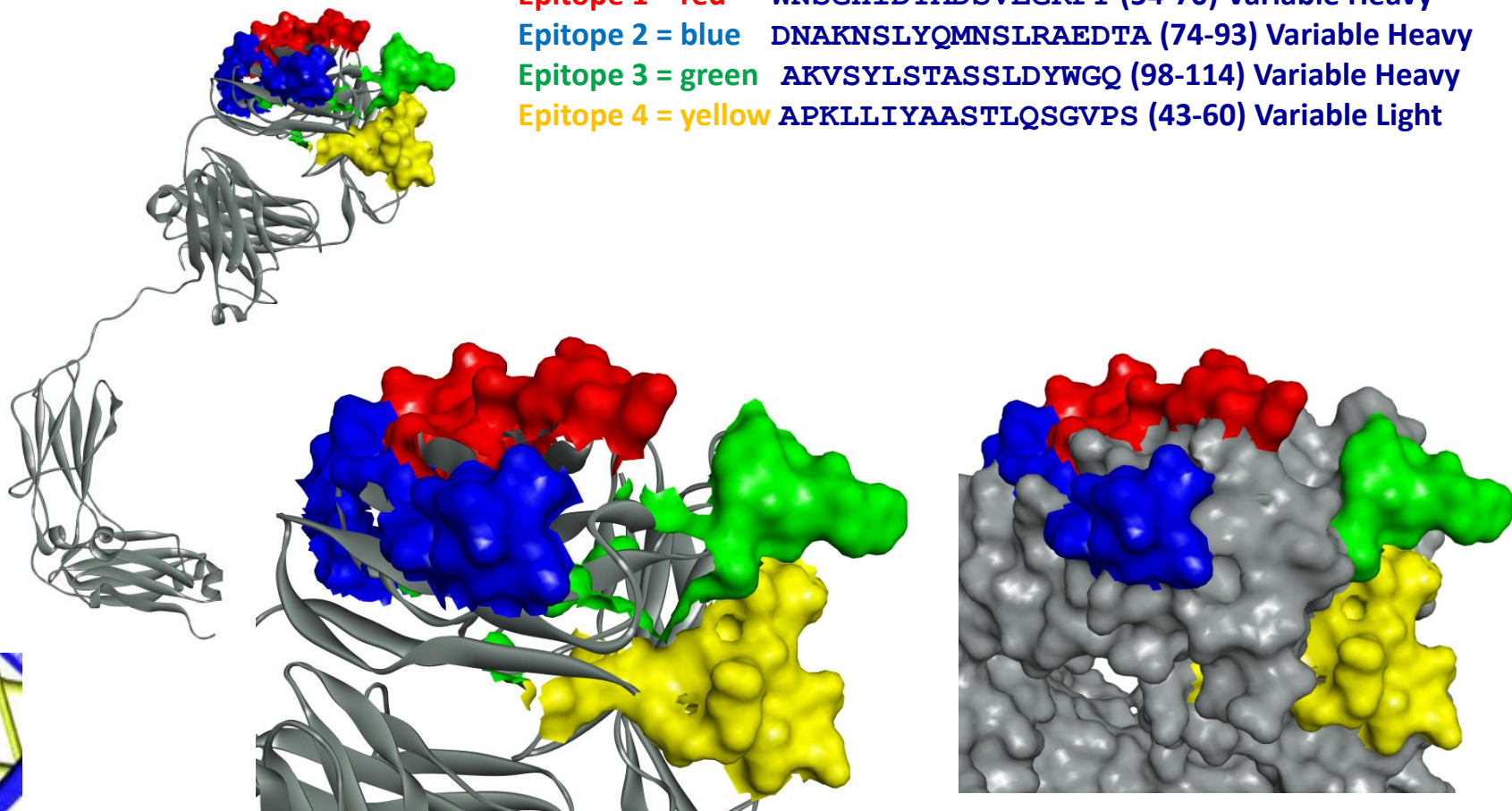
1 10 20 30 40 50 60 70 80 90 100

DIQMTQSPSSLSASVGDRTITCRASQGIRNYLAWYQQKPGKAPKLLIYAAS T LQSGVPSRFSGSGSGTDFTLTISSLQPEDVATYYCQRYNRAPYTFGGQTKVEIK

Four unique regions were identified by ProPresent® (highlighted green). Peptides located around the CDR-2 and CDR-3 of Humira® heavy chain and CDR-2 of the light chain

Humira® Epitope Regions Shown on Homology Model

- Epitope 1 = red WNSGHIDYADSVEGRFT (54-70) Variable Heavy
- Epitope 2 = blue DNAKNSLYQMNSLRAEDTA (74-93) Variable Heavy
- Epitope 3 = green AKVSYLSTASSLDYWGQ (98-114) Variable Heavy
- Epitope 4 = yellow APKLLIYAASTLQSGVPS (43-60) Variable Light



HLA Restriction of Functional T cell epitopes

Analysis of Peptide 102 (SLYLQMNSLRAEDTA) from Humira® Heavy Chain CDR3

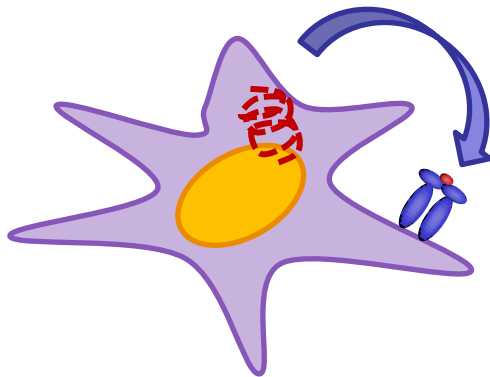
Donor	DRB1	
1	*13:01	*15:01
2	*03:01	*04:04
3	*03:01	*16:01
4	*01:01	*13:01
5	*01:01	*03:01

Unique Peptides	Amino Acid Start/End	Protein Domain	DRB1* Alleles Present with Detected Peptide							Likely Allele Association Based on known Anchors							
			*01:01	*03:01	*04:01	*04:03					*04:01						
APKLLIYAASTLQSGVPS	43-60	Variable Light Chain	*01:01	*03:01	*04:01	*04:03								*04:01			
WNSGHIDYADSVEGRFT	54-70	Variable Heavy Chain	*01:01	*04:03										*01:01			
DNAKNSLYLQMNSLRAEDTA	74-93	Variable Heavy Chain	*01:01	*13:01	*04:01	*04:03	*14:54	*15:01	*15:02	*03:01	*08:01	*01:01	*13:02	*04:01	*03:01		
AKVSYLSTASSLDYWQ	98-114	Variable Heavy Chain	*04:01											*04:01			

ProPresent[®] is Plug and Play

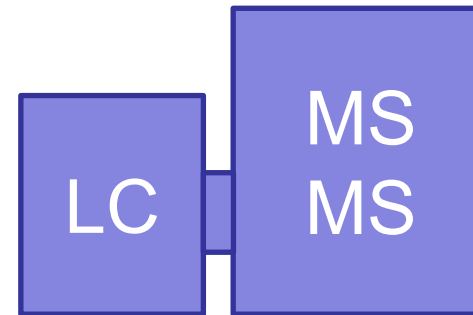
Step 1: completely flexible

- Generate MHC-peptide presenting cells by any means
- pellet
- freeze



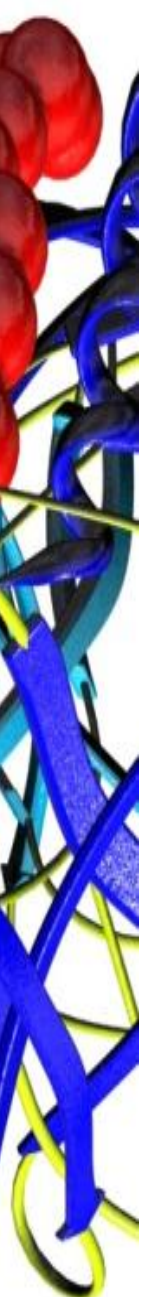
Step 2: highly standardized

- Recover MHC, peptide
- Analyse by LC-MS/MS



ProPresent[®]: Types of Projects to Date

- Factor replacement protein => consistently able to identify same peptides; good comparison to functional assays.
- Haptenated protein => completely changes antigen presentation, presenting haptenated and also new un-haptenated peptides
- Several recombinant antibodies => similar data to those obtained by others in terms of epitopes identified
- Known allergen peptides identified from known allergen protein in protein lysate
- Identification of presented peptides from eukaryotic pathogen => direct drilling into the Immunoepitopeome including dependent on life cycle



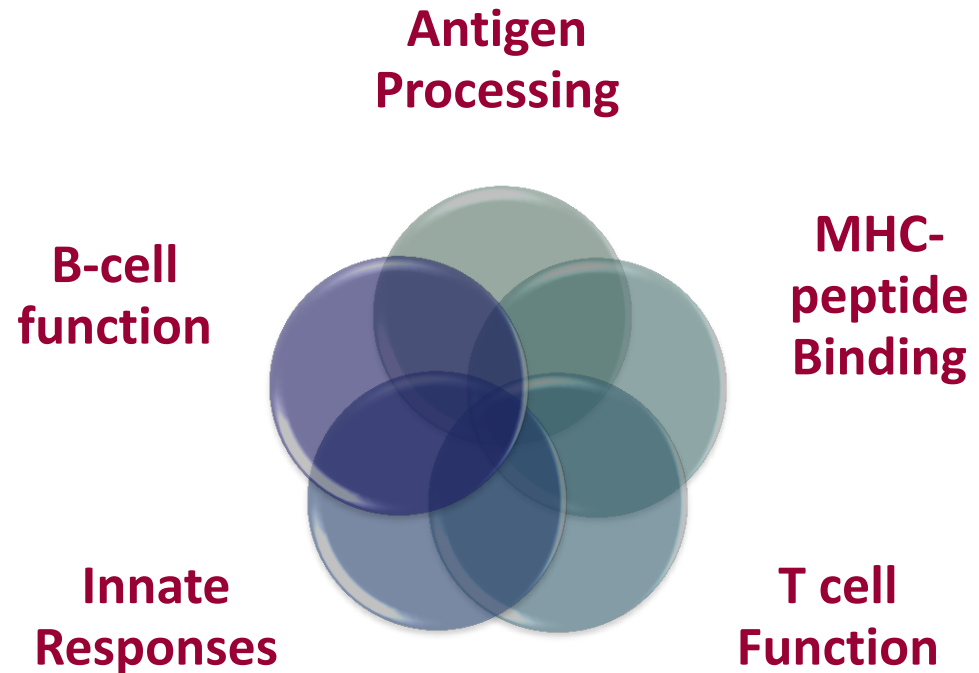
ProPresent® Conclusions

- **Rapid** way to identify sequences of key relevance for the immunogenicity of target protein
- Can answer otherwise confounding questions in a clear and decisive way
- Represents a key element in profiling any protein based product candidate and should be considered for inclusion in any product file
- Unique service – only available at ProImmune



Mastering Immunogenicity Relies on Combination of Several Assays

- ❖ ProPresent®
Antigen Presentation Assay
- ❖ ProImmune Reveal®
In vitro HLA Binding Assays
T cell, DC: T cell assays
- ❖ ProArray Ultra®
UHT Ligand Binding Assay Service
- ❖ ProStorm®
Cytokine Release Assay
- ❖ Pro5® MHC Pentamers
- ❖ Donor-Cohort Sourcing
- ❖ ELISPOT /ICS
- ❖ typeHLA™
- ❖ thinkpeptides®





For further information please contact:

ProlImmune, Inc. (Sarasota, FL)

ProlImmune Limited (Oxford, UK)

enquiries@proimmune.com

US & Canada: (888) 505 7765

All other countries: +44 870 042 7279

www.proimmune.com